The superiority of the present invention over the prior art will be demonstrated using the following experiments.

(First Experiment)

In a first experiment, a comparison experiment was performed using λDNA as a template in order to compare the pseudo-positive repression effects of the allele-specific primers according to the present invention and the prior art allele-specific primers.

The experiment was specifically performed as follows. The following two types of single-stranded DNA sequences were set as the target sequences: (i) 3'-CTACTCAAGCACAGGCATGTTGACC-5', which is a part of a double-stranded DNA sequence consisting of the 7031 through 7155th bases of the λDNA, 5'-GATGAGTTCGTGTCCGTACAACTGG-3'/3'-CTACTCAAGCACAGGCATGTTGACC-5', and (ii) 3'-CTACTCAAGCACAGGCATGTTGACT-5' obtained by converting the 5' terminal base of the sequence (i) into T. (The former ((i)) will be referred to as "Target Sequence 1", and the latter ((ii)) will be referred to as "Target Sequence 2".) A pseudo-positiveness comparison experiment regarding the determination of the 5' terminal base C (of Target Sequence 1) and the 5' terminal base T (of Target Sequence 2) was performed.

The following eight types of allele-specific primers were used for the first experiment (in each sequence, the underlined base is uncomplementary to Target Sequence 1). Among the following primers, (1-1) through (1-3) are prior art allele-specific primers

(having an SNP corresponding base at the 3' terminal and having a base uncomplementary to the target sequences at the third position from the 3' terminal). (1-4) through (1-8) are allele-specific primers according to the present invention (having an SNP corresponding base at the 3' terminal and having bases uncomplementary to the target sequences at the second and third positions from the 3' terminal).

(1-1) 5'-GATGAGTTCGTGTCCGTACAACAGA-3'

(1-2) 5'-GATGAGTTCGTGTCCGTACAACGGA-3'

(1-3) 5'-GATGAGTTCGTGTCCGTACAACCGA-3'

(1-4) 5'-GATGAGTTCGTGTCCGTACAACAAA-3'

(1-5) 5'-GATGAGTTCGTGTCCGTACAACGAA-3'

(1-6) 5'-GATGAGTTCGTGTCCGTACAACATA-3'

(1-7) 5'-GATGAGTTCGTGTCCGTACAACACA-3'

(1-8) 5'-GATGAGTTCGTGTCCGTACAACGCA-3'

(Target Sequence 1) .....ACC-5'

More specifically, in the case of (1-1) through (1-3), the 3' terminal base A is uncomplementary to the 5' terminal base C of Target Sequence 1. The third base from the 3' terminal is different among (1-1) through (1-3) (A in (1-1), G in (1-2), and C in (1-3)), but all these bases are uncomplementary to the base A at the third position from the 5' terminal base of Target Sequence 1.

In the case of (1-4) through (1-8), the 3' terminal base A is uncomplementary to the 5' terminal base C of Target Sequence 1. The second and third bases from the 3' terminal are different among (1-4) through (1-8), but all these bases are respectively uncomplementary to the second and third bases from the 5' terminal base of Target Sequence 1.

Accordingly, all these allele-specific primers are expected to generate an extension reaction with respect to Target Sequence 2, but not to generate an extension reaction with respect to Target Sequence 1. The purpose of the first experiment is to compare the pseudo-positive repression effects. Therefore, PCR was performed as follows using these allele-specific primers as the forward primers and using only the λDNA having Target Sequence 1 as the template.

First, a 20 μL of reaction solution containing the following was prepared.

2 μL of enzyme mixture of LightCycler-FastStart DNA Master SYBER Green I Kit (produced by Roche Diagnostics),

λDNA having 10 μg/mL of Target Sequence 1,

1 μM of forward primer (each of (1-1) through (1-8) allele-specific primers),

1 μM of reverse primer, and

1.6 mM of $MgCl_2$.

Next, each resultant reaction solution was subjected to a PCR reaction using LightCycler (thermal cycler produced by

Roche Diagnostics) under the conditions of denaturation step: 94°C, 10 sec., annealing step: 58°C, 10 sec., extension step: 72°C, 10 sec., and the number of cycles: 30 cycles.

Finally, each PCT reaction result was analyzed using Bioanalyzer 2100 (DNA electrophoresis system produced by Agilent Technologies).

As the reverse primer, a DNA consisting of 5'-GAATCACGGTATCCGGCTGCGCTGA-3' was used. This reverse primer is completely complementary to 5'-TCAGCGCAGCCGGATACCGTGATTC-3', which is a part of a double-stranded DNA sequence consisting of the 7406 through 7430th bases of the λDNA, 5'-TCAGCGCAGCCGGATACCGTGATTC-3'/5'-GAATCACGGTATCCGGCTGCGCTGA-3'. Accordingly, if the reaction had proceeded satisfactorily by the PCR reaction with each of the (1-1) through (1-8) allele-specific primers, a 400 bp DNA amplification product would have been obtained.

The analysis results are shown in FIG. 1.

These results show the concentrations (nM) of the target DNA fragments after the PCR reaction.

It is understood from these results that the concentration was 100 nM or greater when each of the (1-1) through (1-3) allele-specific primers was used as the forward primer, whereas the concentration was several nM or less when each of the (1-4) through (1-8) allele-specific primers was used as the forward primer. The difference is clear, which demonstrates that the

pseudo-positive repression effect of the allele-specific primers according to the present invention is significantly superior to that of the prior art allele-specific primers.

(Second Experiment)

In a second experiment also, like in the first experiment, a comparison experiment was performed using λDNA as a template in order to compare the pseudo-positive repression effects of the allele-specific primers according to the present invention and the prior art allele-specific primers.

The experiment was specifically performed as follows. Target Sequence 1, and 3'-CTACTCAAGCACAGGCATGTTGACA-5'(referred to as Target Sequence 3) obtained by converting the 5' terminal base of Target Sequence 1 into A, were set as the target sequences. A pseudo-positiveness comparison experiment regarding the determination of the 5' terminal base C (of Target Sequence 1) and the 5' terminal base A (of Target Sequence 3) was performed.

The following eight types of allele-specific primers were used for the second experiment (in each sequence, the underlined base is uncomplementary to Target Sequence 1). Among the following primers, (2-1) through (2-3) are prior art allele-specific primers (having an SNP corresponding base at the 3' terminal and having a base uncomplementary to the target sequences at the third position from the 3' terminal). (2-4) through (2-8) are allele-specific primers according to the present invention (having an SNP corresponding base at the 3' terminal

and having bases uncomplementary to the target sequences at the second and third positions from the 3' terminal).

(2-1) 5'-GATGAGTTCGTGTCCGTACAACAGT-3'

(2-2) 5'-GATGAGTTCGTGTCCGTACAACGGT-3'

(2-3) 5'-GATGAGTTCGTGTCCGTACAACCGT-3'

(2-4) 5'-GATGAGTTCGTGTCCGTACAACAAT-3'

(2-5) 5'-GATGAGTTCGTGTCCGTACAACGAT-3'

(2-6) 5'-GATGAGTTCGTGTCCGTACAACCAT-3'

(2-7) 5'-GATGAGTTCGTGTCCGTACAACGTT-3'

(2-8) 5'-GATGAGTTCGTGTCCGTACAACGCT-3'

(Target Sequence 1) .....ACC-5'

More specifically, in the case of (2-1) through (2-3), the 3' terminal base T is uncomplementary to the 5' terminal base C of Target Sequence 1. The third base from the 3' terminal is different among (2-1) through (2-3) (A in (2-1), G in (2-2), and C in (2-3)), but all these bases are uncomplementary to the base A at the third position from the 5' terminal base of Target Sequence 1.

In the case of (2-4) through (2-8), the 3' terminal base T is uncomplementary to the 5' terminal base C of Target Sequence 1. The second and third bases from the 3' terminal are different among (2-4) through (2-8), but all these bases are respectively uncomplementary to the second and third bases from the 5' terminal

base of Target Sequence 1.

Accordingly, all these allele-specific primers are expected to generate an extension reaction with respect to Target Sequence 3, but not to generate an extension reaction with respect to Target Sequence 1. The purpose of the second experiment is to compare the pseudo-positive repression effects. Therefore, PCR was performed as follows using these allele-specific primers as the forward primers and using only the λDNA having Target Sequence 1 as the template.

First, a 20 μL of reaction solution containing the following was prepared.

2 μL of enzyme mixture of LightCycler-FastStart DNA Master SYBER Green I Kit (produced by Roche Diagnostics),

λDNA having 10 μg/mL of Target Sequence 1,

1 μM of forward primer (each of (2-1) through (2-8) allele-specific primers),

1 μM of reverse primer, and

1.6 mM of $MgCl_2$.

Next, each resultant reaction solution was subjected to a PCR reaction using LightCycler (thermal cycler produced by Roche Diagnostics) under the conditions of denaturation step: 94°C, 10 sec., annealing step: 58°C, 10 sec., extension step: 72°C, 10 sec., and the number of cycles: 30 cycles.

Finally, each PCT reaction result was analyzed using Bioanalyzer 2100 (DNA electrophoresis system produced by Agilent

Technologies).

As the reverse primer, a DNA consisting of 5'-GAATCACGGTATCCGGCTGCGCTGA-3' was used. This reverse primer is completely complementary to 5'-TCAGCGCAGCCGGATACCGTGATTC-3', which is a part of a double-stranded DNA sequence consisting of the 7406 through 7430th bases of the λDNA, 5'-TCAGCGCAGCCGGATACCGTGATTC-3'/5'-GAATCACGGTATCCGGCTGCGCTGA-3'. Accordingly, if the reaction had proceeded satisfactorily by the PCR reaction with each of the (2-1) through (2-8) allele-specific primers, a 400 bp DNA amplification product would have been obtained.

The analysis results are shown in FIG. 2.

These results show the concentrations (nM) of the target DNA fragments after the PCR reaction.

It is understood from these results that the concentrations were respectively 238.9 nM, 210.8 nM, and 178.8 nM when the (2-1) through (2-3) allele-specific primers were used as the forward primers, whereas the concentrations were respectively only 0.57 nM, 0.78 nM, 5.7 nM, 1.86 nM, and 18.2 nM when the (2-4) through (2-8) allele-specific primers were used as the forward primers. The difference is clear, which demonstrates that the pseudo-positive repression effect of the allele-specific primers according to the present invention is significantly superior to that of the prior art allele-specific primers.

(Third Experiment)

In a third experiment also, like in the first experiment, a comparison experiment was performed using λDNA as a template in order to compare the pseudo-positive repression effects of the allele-specific primers according to the present invention and the prior art allele-specific primers.

The experiment was specifically performed as follows. The following two types of single-stranded DNA sequences were set as the target sequences: (i) 3'-CCGACTGGGACTACTCAAGCACAGGCATGT-5', which is a part of a double-stranded DNA sequence consisting of the 7121 through 7150th bases of the λDNA, 5'-GGCTGACCCTGATGAGTTCGTGTCCGTACA-3'/3'-CCGACTGGGACTACTCAAGCACAGGCATGT-5', and (ii) 3'-CCGACTGGGACTACTCAAGCACAGGCATGG-5' obtained by converting the 5' terminal base of the sequence (i) into G. (The former ((i)) will be referred to as "Target Sequence 4", and the latter ((ii)) will be referred to as "Target Sequence 5".) A pseudo-positiveness comparison experiment regarding the determination of the 5' terminal base T (of Target Sequence 4) and the 5' terminal base G (of Target Sequence 5) was performed.

The following eight types of allele-specific primers were used for the third experiment (in each sequence, the underlined base is uncomplementary to Target Sequence 4). Among the following primers, (3-1) through (3-3) are prior art allele-specific primers (having an SNP corresponding base at the 3' terminal and having

a base uncomplementary to the target sequences at the third position from the 3' terminal). (3-4) through (3-8) are allele-specific primers according to the present invention (having an SNP corresponding base at the 3' terminal and having bases uncomplementary to the target sequences at the second and third positions from the 3' terminal).

(3-1) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTTCC-3'

(3-2) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTGCC-3'

(3-3) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTCCC-3'

(3-4) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTTTC-3'

(3-5) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTGAC-3'

(3-6) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTGGC-3'

(3-7) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTCTC-3'

(3-8) 5'-GGCTGACCCTGATGAGTTCGTGTCCGTCGC-3'

(Target Sequence 4) .....TGT-5'

More specifically, in the case of (3-1) through (3-3), the 3' terminal base C is uncomplementary to the 5' terminal base T of Target Sequence 4. The third base from the 3' terminal is different among (3-1) through (3-3) (T in (3-1), G in (3-2), and C in (3-3)), but all these bases are uncomplementary to the base T at the third position from the 5' terminal base of Target Sequence 4.

In the case of (3-4) through (3-8), the 3' terminal base

C is uncomplementary to the 5' terminal base T of Target Sequence 4. The second and third bases from the 3' terminal are different among (4-4) through (4-8), but all these bases are respectively uncomplementary to the second and third bases from the 5' terminal base of Target Sequence 4.

Accordingly, all these allele-specific primers are expected to generate an extension reaction with respect to Target Sequence 5, but not to generate an extension reaction with respect to Target Sequence 4. The purpose of the third experiment is to compare the pseudo-positive repression effects. Therefore, PCR was performed as follows using these allele-specific primers as the forward primers and using only the λDNA having Target Sequence 4 as the template.

First, a 20 μL of reaction solution containing the following was prepared.

2 μL of enzyme mixture of LightCycler-FastStart DNA Master SYBER Green I Kit (produced by Roche Diagnostics),

λDNA having 10 μg/mL of Target Sequence 4,

1 μM of forward primer (each of (3-1) through (3-8) allele-specific primers),

1 μM of reverse primer, and

1.6 mM of $MgCl_2$.

Next, each resultant reaction solution was subjected to a PCR reaction using LightCycler (thermal cycler produced by Roche Diagnostics) under the conditions of denaturation step: 94°C,

10 sec., annealing step: 58°C, 10 sec., extension step: 72°C, 10 sec., and the number of cycles: 30 cycles.

Finally, each PCT reaction result was analyzed using Bioanalyzer 2100 (DNA electrophoresis system produced by Agilent Technologies).

As the reverse primer, a DNA consisting of 5'-GAATCACGGTATCCGGCTGCGCTGA-3' was used. This reverse primer is completely complementary to 5'-TCAGCGCAGCCGGATACCGTGATTC-3', which is a part of a double-stranded DNA sequence consisting of the 7406 through 7430th bases of the λDNA, 5'-TCAGCGCAGCCGGATACCGTGATTC-3'/5'-GAATCACGGTATCCGGCTGCGCTGA-3'. Accordingly, if the reaction had proceeded satisfactorily by the PCR reaction with each of the (3-1) through (3-8) allele-specific primers, a 310 bp DNA amplification product would have been obtained.

The analysis results are shown in FIG. 3.

These results show the concentrations (nM) of the target DNA fragments after the PCR reaction.

It is understood from these results that the concentration was 110 nM or greater when each of the (3-1) through (3-3) allele-specific primers was used as the forward primer, whereas the concentration was 20 nM or less when each of the (3-4) through (3-8) allele-specific primers was used as the forward primer. The difference is clear, which demonstrates that the pseudo-positive repression effect of the allele-specific primers

according to the present invention is significantly superior to that of the prior art allele-specific primers.

(Fourth Experiment)

In a fourth experiment, like in the first experiment, a comparison experiment was performed using λDNA as a template in order to compare the pseudo-positive repression effects of the allele-specific primers according to the present invention and the prior art allele-specific primers.

The experiment was specifically performed as follows. The following two types of single-stranded DNA sequences were set as the target sequences: (i) 3'-CAGGCATGTTGACCGCATTAGTAC-5', which is a part of a double-stranded DNA sequence consisting of the 7141 through 7165th bases of the λDNA, 5'-GTCCGTACAACTGGCGTAATCATG-3'/3'-CAGGCATGTTGACCGCATTAGTAC-5', and (ii) 3'- CAGGCATGTTGACCGCATTAGTAT-5' obtained by converting the 5' terminal base of the sequence (i) into T. (The former ((i)) will be referred to as "Target Sequence 6", and the latter ((ii)) will be referred to as "Target Sequence 7".) A pseudo-positiveness comparison experiment regarding the determination of the 5' terminal base C (of Target Sequence 6) and the 5' terminal base T (of Target Sequence 7) was performed.

The following eight types of allele-specific primers were used for the fourth experiment (in each sequence, the underlined base is uncomplementary to Target Sequence 6). Among the following primers, (4-1) through (4-3) are prior art

allele-specific primers (having an SNP corresponding base at the 3' terminal and having a base uncomplementary to the target sequences at the third position from the 3' terminal). (4-4) through (4-8) are allele-specific primers according to the present invention (having an SNP corresponding base at the 3' terminal and having bases uncomplementary to the target sequences at the second and third positions from the 3' terminal).

(4-1) 5'-GTCCGTACAACTGGCGTAATCTTA-3'

(4-2) 5'-GTCCGTACAACTGGCGTAATCGTA-3'

(4-3) 5'-GTCCGTACAACTGGCGTAATCCTA-3'

(4-4) 5'-GTCCGTACAACTGGCGTAATCTAA-3'

(4-5) 5'-GTCCGTACAACTGGCGTAATCTGA-3'

(4-6) 5'-GTCCGTACAACTGGCGTAATCTCA-3'

(4-7) 5'-GTCCGTACAACTGGCGTAATCGAA-3'

(4-8) 5'-GTCCGTACAACTGGCGTAATCCCA-3'

(Target Sequence 6) .....TAC-5'

More specifically, in the case of (4-1) through (4-3), the 3' terminal base A is uncomplementary to the 5' terminal base C of Target Sequence 6. The third base from the 3' terminal is different among (4-1) through (4-3) (T in (4-1), G in (4-2), and C in (4-3)), but all these bases are uncomplementary to the base T at the third position from the 5' terminal base of Target Sequence 6.

In the case of (4-4) through (4-8), the 3' terminal base A is uncomplementary to the 5' terminal base C of Target Sequence 6. The second and third bases from the 3' terminal are different among (4-4) through (4-8), but all these bases are respectively uncomplementary to the second and third bases from the 5' terminal base of Target Sequence 6.

Accordingly, all these allele-specific primers are expected to generate an extension reaction with respect to Target Sequence 7, but not to generate an extension reaction with respect to Target Sequence 6. The purpose of the fourth experiment is to compare the pseudo-positive repression effects. Therefore, PCR was performed as follows using these allele-specific primers as the forward primers and using only the λDNA having Target Sequence 6 as the template.

First, a 20 μL of reaction solution containing the following was prepared.

2 μL of enzyme mixture of LightCycler-FastStart DNA Master SYBER Green I Kit (produced by Roche Diagnostics),

λDNA having 10 μg/mL of Target Sequence 6,

1 μM of forward primer (each of (4-1) through (4-8) allele-specific primers),

1 μM of reverse primer, and

1.6 mM of $MgCl_2$.

Next, each resultant reaction solution was subjected to a PCR reaction using LightCycler (thermal cycler produced by

Roche Diagnostics) under the conditions of denaturation step: 94°C, 10 sec., annealing step: 58°C, 10 sec., extension step: 72°C, 10 sec., and the number of cycles: 30 cycles.

Each solution after the PCR reaction was electrophoresis-treated with 1% agarose gel and then the intensity of fluorescence derived from the target DNA amplification product was analyzed using Microarray Scanner FLA8000 produced by Fuji Photo Film Co., Ltd.

As the reverse primer, a DNA consisting of 5'-GGTTATCGAAATCAGCC-3' was used. This reverse primer is completely complementary to 5'-GGCTGATTTCGATAACC-3', which is a part of a double-stranded DNA sequence consisting of the 7608 through 7630th bases of the λDNA, 5'-GGCTGATTTCGATAACC-3'/5'-GGTTATCGAAATCAGCC-3'. Accordingly, if the reaction had proceeded satisfactorily by the PCR reaction with each of the (4-1) through (4-8) allele-specific primers, a 490 bp DNA amplification product would have been obtained.

The analysis results are shown in FIG. 4.

FIG. 4 shows the intensity of fluorescence derived from the target DNA amplification product obtained with each allele-specific primer, where the intensity of fluorescence derived from the target DNA amplification product obtained with the (4-3) allele-specific primer as the forward primer is set as 100%.

It is understood from these results that the percentages

of the intensity of fluorescence were respectively 98.3%, 91.8%, and 100% when the (4-1) through (4-3) allele-specific primers were used as the forward primers, whereas the percentages of the intensity of fluorescence were respectively only 0.48%, 1.01%, 0% (not detected), 0% (not detected), and 0% (not detected) when the (4-4), (4-5), (4-6), (4-7) and (4-8) allele-specific primers were used as the forward primers. The difference is clear, which demonstrates that the pseudo-positive repression effect of the allele-specific primers according to the present invention is significantly superior to that of the prior art allele-specific primers.

(Fifth Experiment)

In a fifth experiment, like in the first experiment, a comparison experiment was performed using λDNA as a template in order to compare the pseudo-positive repression effects of the allele-specific primers according to the present invention and the prior art allele-specific primers.

The experiment was specifically performed as follows. The following two types of single-stranded DNA sequences were set as the target sequences: (i) 3'-CTCCTCAGGTACTGCTTT-5', which is a part of a double-stranded DNA sequence consisting of the 7193 through 7210th bases of the λDNA, 5'-GAGGAGTCCATGACGAAA-3'/3'-CTCCTCAGGTACTGCTTT-5', and (ii) 3'-CTCCTCAGGTACTGCTTC-5' obtained by converting the 5' terminal base of the sequence (i) into C. (The former ((i)) will

be referred to as "Target Sequence 8", and the latter ((ii)) will be referred to as "Target Sequence 9".) A pseudo-positiveness comparison experiment regarding the determination of the 5' terminal base T (of Target Sequence 8) and the 5' terminal base C (of Target Sequence 9) was performed.

The following eight types of allele-specific primers were used for the fifth experiment (in each sequence, the underlined base is uncomplementary to Target Sequence 8). Among the following primers, (5-1) through (5-3) are prior art allele-specific primers (having an SNP corresponding base at the 3' terminal and having a base uncomplementary to the target sequences at the third position from the 3' terminal). (5-4) through (5-8) are allele-specific primers according to the present invention (having an SNP corresponding base at the 3' terminal and having bases uncomplementary to the target sequences at the second and third positions from the 3' terminal).

(5-1) 5'-GAGGAGTCCATGACGTAG-3'

(5-2) 5'-GAGGAGTCCATGACGGAG-3'

(5-3) 5'-GAGGAGTCCATGACGCAG-3'

(5-4) 5'-GAGGAGTCCATGACGGTG-3'

(5-5) 5'-GAGGAGTCCATGACGCTG-3'

(5-6) 5'-GAGGAGTCCATGACGTGG-3'

(5-7) 5'-GAGGAGTCCATGACGTCG-3'

(5-8) 5'-GAGGAGTCCATGACGCCG-3'

(Target Sequence 8) .....TTT-5'

More specifically, in the case of (5-1) through (5-3), the 3' terminal base G is uncomplementary to the 5' terminal base T of Target Sequence 8. The third base from the 3' terminal is different among (5-1) through (5-3) (T in (5-1), G in (5-2), and C in (5-3)), but all these bases are uncomplementary to the base T at the third position from the 5' terminal base of Target Sequence 8.

In the case of (5-4) through (5-8), the 3' terminal base G is uncomplementary to the 5' terminal base T of Target Sequence 8. The second and third bases from the 3' terminal are different among (5-4) through (5-8), but all these bases are respectively uncomplementary to the second and third bases from the 5' terminal base of Target Sequence 8.

Accordingly, all these allele-specific primers are expected to generate an extension reaction with respect to Target Sequence 9, but not to generate an extension reaction with respect to Target Sequence 8. The purpose of the fifth experiment is to compare the pseudo-positive repression effects. Therefore, PCR was performed as follows using these allele-specific primers as the forward primers and using only the λDNA having Target Sequence 8 as the template.

First, a 20 μL of reaction solution containing the following was prepared.

2 μL of enzyme mixture of LightCycler-FastStart DNA Master SYBER Green I Kit (produced by Roche Diagnostics),

λDNA having 10 μg/mL of Target Sequence 8,

1 μM of forward primer (each of (5-1) through (5-8) allele-specific primers),

1 μM of reverse primer, and

1.6 mM of $MgCl_2$.

Next, each resultant reaction solution was subjected to a PCR reaction using LightCycler (thermal cycler produced by Roche Diagnostics) under the conditions of denaturation step: 94°C, 10 sec., annealing step: 58°C, 10 sec., extension step: 72°C, 10 sec., and the number of cycles: 30 cycles.

Each solution after the PCR reaction was electrophoresis-treated with 1% agarose gel and then the intensity of fluorescence derived from the target DNA amplification product was analyzed using Microarray Scanner FLA8000 produced by Fuji Photo Film Co., Ltd.

As the reverse primer, a DNA consisting of 5'-GGTTATCGAAATCAGCC-3' was used. This reverse primer is completely complementary to 5'-GGCTGATTTCGATAACC-3', which is a part of a double-stranded DNA sequence consisting of the 7608 through 7630th bases of the λDNA, 5'-GGCTGATTTCGATAACC-3'/5'-GGTTATCGAAATCAGCC-3'. Accordingly, if the reaction had proceeded satisfactorily by the PCR reaction with each of the (5-1) through (5-8) allele-specific primers, a

438 bp DNA amplification product would have been obtained.

The analysis results are shown in FIG. 5.

FIG. 5 shows the intensity of fluorescence derived from the target DNA amplification product obtained with each allele-specific primer, where the intensity of fluorescence derived from the target DNA amplification product obtained with the (5-3) allele-specific primer as the forward primer is set as 100%.

It is understood from these results that the percentages of the intensity of fluorescence were respectively 53.7%, 64.8%, and 100% when the (5-1) through (5-3) allele-specific primers were used as the forward primers, whereas the percentages of the intensity of fluorescence were respectively only 0% (not detected), 0% (not detected), 0% (not detected), 1.2%, and 2.2% when the (5-4), (5-5), (5-6), (5-7) and (5-8) allele-specific primers were used as the forward primers. The difference is clear, which demonstrates that the pseudo-positive repression effect of the allele-specific primers according to the present invention is significantly superior to that of the prior art allele-specific primers.